Calculation of the Circular Dichroism of *Chironomus* Hemoglobin in the Light of the Quality of Its X-Ray Structure

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For the larval hemoglobin III of the insect *Chironomus thummi thummi*, the rotational strength of the B (Soret) and Q band is calculated with a monopole/monopole and a dipole/dipole approximation applied to the atomic co-ordinates of the X-ray structures at 2.5 and 1.4 Å resolution.

In previous calculations using the 2.5 Å co-ordinates and with perturbing groups being restricted to aromatic side-chains, the dipole/dipole approximation clearly confirmed the negative sign observed by experiment. The predominant interactions were those of phenylalanine residues.

Recalculation with X-ray data refined to the present limits of performance corroborates the negativity of the rotational strength, but now the most important contributions are due to the peptide bonds formerly neglected. Also for the 2.5 Å coordinates it is learned that in contrast to earlier expectation the influence of the backbone in this hemoglobin is rather strong. A substantial contribution is further obtained for the perturbation by one of the propionic acid carboxylate groups of the heme.

Perspectives and problems of the approach are outlined.

Introduction

In spite of the complexity of protein structures, there have been promising attempts to calculate the circular dichroism of selected chromophores by applying certain quantum chemical mechanisms to the geometry described by the atomic co-ordinates of their X-ray analyses (see references [1–9]).

Woody and coworkers pioneered the calculation of the rotational strength of heme bands in myoglobin and hemoglobin (Woody and Hsu [1–3]). These calculations successfully predicted the positive sign of these Cotton effects on the basis of a coupling mechanism between the π systems of the heme and of the aromatic amino acid side-chains. For the larval hemoglobin III of the insect *Chironomus thummi thummi*, the sign of the Soret Cotton effect was found to be negative (Formanek and Engel [10]). When in 1971 the X-ray data for this hemoglobin became available (Huber *et al.* [11]), it was understood to be a challenging case to test the capability of the method to correctly predict the observed inversion of sign. It was shown that a dipole/dipole coupling mechanism (Kirkwood [12]) was indeed adequate and that the main contributions responsible for this effect were due to phenylalanine residues in functional positions normally occupied by aliphatic amino acids.

In 1975 it was confirmed by Woody [7] that for lamprey hemoglobin (Hendrickson *et al.* [13]), another case with negative heme Cotton effects (Sugita *et al.* [14)], the sign inversion can also be explained by the influence of aromatic side-chains.

Recently the X-ray structure analysis of *Chironomus* hemoglobin was extended from 2.5 to 1.4 Å resolution and refined to an average R-value of about 0.20 (Steigemann *et al.* [15]). With respect to the as yet poor experience with protein CD calculations, we felt that the existence of data which have been improved to the present limits of performance obliged us to again check the reliability of the results.

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The calculations reported in this communication are not a mere repetition with the new structural data, but include

1) atomic co-ordinates at 2.5 and 1.4 Å resolution,
2) different approximations for the perturbation potential,
3) extension of the number and nature of perturbed heme transitions and
4) extension of the number and nature of perturbing groups.

Methods

The rotational strength of the B (Soret) and the Q band of the heme was calculated according to the expression given by Tinoco, Jr. [16]. The interaction potential in this expression was approximated by two different methods, the dipole/dipole approximation of Kirkwood's [12] and the electrostatic monopole/monopole approximation of Woody's [17].

The quantitative characteristics for the π-π^* transitions of the single perturbing groups such as transition moments, frequencies, and monopoles were taken from the literature (Hsu and Woody [3], Strickland [4]). In contrast to our previous calculations (Fleischhauer and Wollmer [5]) the orientation of the transition moments within the various groups were now deduced from the monopole distributions.

Results and Discussion

The contributions of perturbing π systems to the rotational strength of the Soret band as calculated with different approximations and atomic co-ordinates at 2.5 and 1.4 Å resolution are listed in Table I.

A result directly comparable to that of our former calculations (Fleischhauer and Wollmer [5]) is the sum of aromatic contributions from the *dipole/dipole* approximation applied to the *2.5 Å* co-ordinates, since previously only aromatic side-chains had been taken into account. The values are in good agreement, the remaining difference being due to slight differences in the orientation of transition moments determined with different procedures (see Methods).

If, for the improved X-ray data at *1.4 Å* resolution, the perturbing groups are likewise restricted to the aromatic side-chains, the absolute value of the rotational strength is so small that it could not be accepted as unequivocal evidence of the negative sign. This point suffices to demonstrate how important it was to reexamine the situation.

Table I. Contributions of different classes of perturbing groups to the rotational strength of the B (Soret) band of *Chironomus* hemoglobin III. Calculations with two different approximations for different resolutions of X-ray crystallographic analysis.

1 DBM = 0.9273×10^{-38} cgs units
= 3.105×10^{-53} SI units [$A^2\ m^3\ sec$].

Class of perturbing groups	Rotational strength [DBM] calculated with approximation			
	m/m	d/d	m/m	d/d
His	−0.036	−0.020	0.048	0.044
Phe	−0.292	−0.203	−0.132	−0.074
Tyr	0.021	0.021	0.030	0.033
Trp	−0.054	−0.041	−0.024	−0.035
Sum-aromatic groups	−0.361	−0.242	−0.078	−0.032
Asp/Glu	0.009	0.010	0.008	0.009
Asn/Gln	0.070	0.071	−0.016	−0.019
Pept. bonds	−0.101	−0.099	−0.194	−0.195
Coo (heme)	−0.126	−0.137	−0.044	−0.055
Sum-other groups	−0.148	−0.155	−0.246	−0.260
Total sum	−0.509	−0.397	−0.324	−0.292
Resolution of X-ray analysis	2.5 Å		1.4 Å	

The negative sign is fully established if further perturbing groups are taken into account. The even higher negative contributions reveal that the limitation of perturbing groups to aromatic side-chains has been arbitrary. For both sets of co-ordinates, a substantial amount of rotational strength is due to peptide bonds and to the propionic acid carboxylate groups of the heme.

As to the *monopole/monopole* approximation, Hsu's and Woody's calculations [1−3, 7] were based on atom-centered monopoles whereas we used an improved approximation of Woody's [18]. In this version, the monopole, centered at each atom, is divided into two which are located one above, the other below the molecular plane of the chromophoric groups. For the *2.5 Å* co-ordinates, the influence of phenylalanine residues is even more pronounced than in the dipole case. This is due to contributions of phenylalanine side-chains close to the heme (Phe F 4 (83) *e. g.*, see Table II). The monopole approximation is known to be more adequate for interactions over short distances. For the *1.4 Å* co-ordinates the reduced phenylalanine contribution is mainly

Table II. Most important single contributions to the rotational strength of the B (Soret) band of *Chironomus* hemoglobin III. Calculations with two different approximations for different resolutions of X-ray crystallographic analysis.
1 DBM = 0.9273×10^{-38} cgs units = 3.105×10^{-53} SI units [$A^2\,m^3\,sec$].

Perturbing Group	Position [19], [20]	Distance [Å] **	Rotational strength [DBM] calculated with approximation				Distance [Å] **
			m/m	d/d	m/m	d/d	
Phe	E 14 (65)	8.9	−0.195	−0.171	−0.145	−0.153	9.5
Phe	E 15 (66)	11.3	−0.092	−0.086	−0.093	−0.097	11.5
Phe	F 4 (83)	5.7	0.034	0.060	−0.059	−0.037	6.3
Phe	H 14 (128)	10.6	0.077	0.077	0.104	0.105	10.6
Phe	H 15 (129)	11.5	−0.080	−0.074	−0.066	−0.069	11.2
His *	E 5 (56)	16.1	−0.015	−0.016			
His *	E 7 (58)				0.049	0.058	8.9
PB	C 3 (33−34)	10.3	−0.008	−0.021	−0.056	−0.055	10.5
PB	E 8 (59−60)	9.6	0.047	0.060	0.040	0.054	9.9
PB	E 9 (60−61)	9.6	−0.046	−0.051	−0.050	−0.055	10.1
COO	Heme (C 7)	5.7	−0.102	−0.110	−0.087	−0.100	5.8
Resolution of analysis			2.5 Å			1.4 Å	

* For explanation see text.
** Distance between the mass centres of the heme and each perturbing group.

responsible for the much smaller absolute value of the total rotational strength. This implies certain changes in the mutual orientation of the heme and phenylalanine side-chains upon refinement of the structure. Peptide bonds have now become at least as powerful a group of contributors to negative ellipticity.

The rotational strength of the Soret band of −0.324 DBM calculated with the monopole/monopole approximation for the structure at 1.4 Å resolution compares reasonably with the experimental value of −0.5 DBM.

Table II contains the most important single contributions. The big changes for Phe F 4 (83) have already been mentioned. It should be noted that some time after completion of the X-ray structure at 2.5 Å resolution the sequence was corrected in that His E 5 (56) and Glu E 7 (58) had to be exchanged in position (Wollmer *et al.* [20], Buse *et al.* [21]). In its revised position, His E 7 has become a substantial positive contributor. Groups that had been neglected in the previous calculations are now encountered in the list of the most prominent contributors. These are certain peptide bonds as well as the carboxylate groups of the heme. The latter were treated as perturbing groups as were the vinyl groups, the distributions of which turned out to have only negligeable influence in their actual positions. There has been discussion on whether the heme were incorporated in the globin in two alternative orientations (Formanek and Engel [11], Steigemann *et al.* [15], La Mar *et al.* [22]), related by a 180° rotation about an axis going through the α and γ methene groups. Since this rotation affects the relative orientation of heme and globin only with respect to the methyl and vinyl groups it should be irrelevant for the rotational strength.

For the rotational strength of the Q band the values of single contributions as well as that of the total sum are throughout very small (see Table III and note the factor of 10^2). The negativity which is found in the case of the 1.4 Å co-ordinates is thus more or less insignificant. This is in total agreement with the CD spectrum in the visible wavelength range, which is hardly detached from the baseline in the negative direction (Gersonde *et al.* [23]).

Concluding Remarks

The interest in the calculation of protein circular dichroism is manifold. It is attempted to provide a causal understanding of the origin of the observed bands. Simultaneously, the overall information of a CD spectrum would be resolved into discrete contributions of specific pairwise interactions. The identification of the main contributors could be useful for the interpretation of conformational changes and an indication of sites for possible chemical modification [9].

However, these calculations remain a difficult task. Short-comings are due to both, inevitable

Table III. Contributions of different classes of perturbing groups to the rotational strength of the Q band of *Chironomus* hemoglobin III. Calculations with two different approximations for different resolutions of X-ray crystallographic analysis.

1 DBM = 0.9273×10^{-38} cgs units
= 3.105×10^{-53} SI units [$A^2\,m^3\,sec$].

Class of perturbing groups	Rotational strength [DBM] $\cdot 10^{+2}$ calculated with approximation			
	m/m	d/d	m/m	d/d
His	−0.13	−0.15	−0.16	0.02
Phe	2.37	0.15	0.08	−0.43
Tyr	−0.07	0.0	0.02	0.01
Trp	0.01	−0.04	0.01	−0.02
Sum-aromatic groups	2.18	−0.04	−0.05	−0.42
Asp/Glu	0.08	−0.04	−0.17	−0.12
Asn/Gln	0.06	0.01	0.0	0.0
Pept. bonds	−1.36	−0.07	−0.12	−0.03
Coo (heme)	−0.37	0.03	0.02	−0.01
Sum-other groups	−1.59	−0.07	−0.27	−0.16
Total sum	0.59	−0.11	−0.32	−0.58
Resolution of X-ray analysis	2.5 Å		1.4 Å	

simplifications in the quantum chemical treatment as well as remaining inaccuracies of the structure. The success of the calculations can only be assessed by their quantitative agreement with the circular dichroism observed in experiment.

This work critically examines the influence of the quality of the structure as it increases with resolution and refinement. For the 2.5 Å co-ordinates, application of the same approximation (dipole/dipole) to the same set of perturbing groups (the aromatic side-chains) clearly confirms the observed negative sign. For the 1.4 Å co-ordinates, however, unequivocal negativity is established only after extension of the perturbing groups to include the peptide bonds. Furthermore, the total rotational strength obtained with the monopole/monopole approximation for the 2.5 Å co-ordinates is much closer to the experimental value than is that for the improved co-ordinates at 1.4 Å resolution. General experience with, for example, hemoglobin, ribonuclease and especially insulin where multiple aggregation states were examined, supports the view that the sign and the order of magnitude can be calculated. Quantitative improvements will certainly require the use of the most accurate crystal co-ordinates.

The remaining difficulties would then be thermal mobility and, with no straightforward issue, possible differences of the structure in the crystal and in solution.

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